Abstract

This review emphasizes some of the challenges and benefits of in vivo imaging of the small animal lung. Because mechanical ventilation plays a key role in high-quality, high-resolution imaging of the small animal lung, the article focuses particularly on the problems of ventilation support, control of breathing motion and lung volume, and imaging during different phases of the breathing cycle. Solutions for these problems are discussed primarily in relation to magnetic resonance imaging, both conventional proton imaging and the newer, hyperpolarized helium imaging of pulmonary airways. Examples of applications of these imaging solutions to normal and diseased lung are illustrated in the rat and guinea pig. Although difficult to perform, pulmonary imaging in the small animal can be a valuable source of information not only for the normal lung, but also for the lung challenged by disease.

Key Words: lung; magnetic resonance imaging; mechanical ventilation; pulmonary; rodent; thorax; ventilator

Introduction

The small animal is an important subject for models of pulmonary disease (Crapo et al. 1992; Gardner et al. 1993), and in vivo imaging of the lung is an important tool for many of these studies (Cutillo 1996). However, the lung is a difficult organ to image in vivo, especially in the small animal. There are two major challenges to imaging the lungs: they are in almost constant motion and the tissue density is low, thus there is little substance for x-ray attenuation or proton signal for magnetic resonance (MR) imaging. Lung imaging is also complicated by the incessant motion of the heart. Because of the importance of the small animal in pulmonary research, we have devoted considerable effort over the past 15 yr to developing noninvasive, nondestructive methods for in vivo lung imaging in the small animal. For this work, we have used two types of MR studies: the more conventional method using proton (1H) imaging and the more recently developed method using hyperpolarized 3He imaging for pulmonary gas spaces. Although the emphasis in this review is on ventilation support and control for MR imaging of the lung, many of the same issues and solutions apply to other noninvasive imaging modalities such as conventional x-ray, microcomputed tomography, single photon emission computed tomography, and positron emission tomography.

Mechanical ventilation is important for in vivo studies in small animals for several reasons. Ventilation provides a method for maintaining proper gas exchange, especially when respiratory depressant anesthetics are used. It provides a convenient way to administer easily controllable gaseous anesthetics. It also provides a way to synchronize imaging to the breathing cycle to significantly diminish the deleterious effects of breathing motion on resolution of images of both the thorax and the upper abdomen. Finally, using synchronous ventilation, imaging data can be captured from the lung during specific phases of the breathing cycle, which is especially important in studies using special gases such as hyperpolarized helium and xenon. This brief review focuses on the following: (1) use of mechanical ventilation for motion control for lung imaging, (2) synchronizing image data acquisition to selected phases of the breathing cycle, and (3) examples of application of these techniques to magnetic resonance imaging of pulmonary models in small animals.

Control of Breathing Motion for Lung Imaging

Breathing motion typically produces partial volume blurring and artifacts when samples are imaged over a significant part of the breath cycle or multiple cycles. Severity of motion degrading effects are dependent on rate and amplitude of breathing motion, imaging frequency, and, of course, spatial resolution. The deleterious effects of breathing motion are typically minimized, especially in the clinical setting, by having subjects hold their breath after a deep inspiration. A variation on this strategy can be used with the anesthetized small animal on mechanical ventilation. In Figure 1 (left), image artifacts that occur when MR acquisition occurs during spontaneous breathing of an anesthetized rat are shown. The weaker secondary images (ghost artifacts) reflect the primary image and are related to phase differences in the raw data space (Fourier) inasmuch as the structures occupy different positions during the breath cycle (Wood and Henkelman 1985). These artifacts, combined with image blurring, almost completely obscure anatomic detail. For example, also in Figure 1 (right) is an...
Figure 1  MR images of the upper abdomen of an anesthetized rat acquired during spontaneous breathing (left) and synchronous with breathing during end-expiration (right) reveal the clearly defined body wall, spinal cord, and abdominal detail. DA, descending aorta; AD, left adrenal gland, with stomach below, and inferior vena cava (IVC) as it courses through the liver with hepatic vasculature clearly showing. Reprinted with permission from Hedlund LW, Johnson GA, Mills GI. 1986b. Magnetic resonance microscopy of the rat thorax and abdomen. Invest Radiol 21:843–846.

Figure 2  Spin echo images from an anesthetized dog obtained during ventilation at 16 breaths per min, about 300 cc per breath (left) and during high-frequency ventilation at 1620 breaths per min (27 Hz), about 3 cc per breath (right). Reprinted with permission from Hedlund LW, Deitz J, Nassar R, Herfkens R, Vock P, Dahlke J, Kubek R, Effmann E, Putman C. 1986a. A ventilator for magnetic resonance imaging. Invest Radiol 21:18-23.
image obtained while using the same imaging sequence except the image data was acquired only when the lungs were at end expiratory volume, at a time when there is little lung motion and when the lungs are at their highest tissue density. The improvement in image resolution is clear because many of the anatomic details of the upper abdomen are easily identified.

There are several ways to synchronize imaging to the breathing cycle. In the example described above (Figure 1), the ventilator generated a pulse on each breath, which in turn triggered imaging. Some commercial small animal ventilators can generate an output signal to trigger external devices. Alternatively, a signal can be detected from the chest, such as from a chest bellows, and this signal can be used to generate a signal for imaging (Ehman et al. 1984). This method is useful for studies with spontaneous breathing animals.

Another approach to minimizing the image-degrading effects of breathing motion is to lessen the amplitude of motion by using very small tidal volumes. This approach can be used with high-frequency (HF) jet ventilation, which utilizes tidal volumes that are a fraction of conventional volumes delivered at high rates (up to 25 Hz) (Froese and Bryan 1981; Rehder et al. 1983). Very small tidal volumes minimize the partial volume blurring and, at high frequencies, normal gas exchange is maintained. We explored this approach early in our attempts to control breathing motion blurring (Hedlund et al. 1986a).

Figure 2 is a comparison between ghosting artifacts during normal ventilation (left, 16 breaths/min, about 300 cc/breath) and those occurring during HF ventilation (right, 1620 breaths/min, about 3 cc/breath). Although ghosting artifacts are less severe with HF ventilation, there is sufficient image degradation to make this unacceptable. This comparison of the impact of two types of breathing motion indicates that ghosting artifacts in MR are not just dependent on the magnitude of motion but also on its frequency in relation to imaging frequency. Although this method is not appropriate

![Figure 3](image-url)
for MR imaging of the lung, it may be useful for other imaging modalities that have lower spatial resolution and a lower susceptibility to motion artifacts. As seen in Figure 2 (right), HF ventilation does minimize motional blurring of other thoracic structures such as descending aorta, azygous vein, pulmonary vessels, vena cava, and chest wall.

For small animal lung imaging in our laboratory, we have used a form of scan synchronous ventilation for both the normal and disease models. We use a custom-built ventilator that is MR compatible. Some commercially available small animal ventilators offer external triggering for synchronizing ventilation with external events such as imaging, and one, which we know of, is MR compatible (CWE, Inc, Ardmore, Pennsylvania). These commercial systems do not offer flexibility of breathing cycle control required for many types of lung studies needed for MR imaging and spectroscopy. To use the typical commercial ventilators with MR, it is necessary to place them at a safe distance from the magnet and therefore some distance from the animal. This is possible with long hoses (several meters) between the breathing gas sources and the animal. However, long hoses can create troublesome dead space problems and significant transit times delays for inspiration and expiration. To avoid these problems, we designed a MR-compatible ventilator that can be used with small animals (e.g., mice to large guinea pigs). A key component of this ventilator is a pneumatically controlled plastic breathing valve that attaches directly to the endotracheal tube, thus eliminating the problem of large gas volumes in ventilator hoses between inspiratory and expiratory valves and thereby decreasing dead space. The nature of the design and control of this valve allows us to generate a wide variety of breathing patterns that can be accurately synchronized to image acquisition. The current ventilator is based on one described earlier (Hedlund et al. 1986a) and has been subsequently modified for the small animal (Hedlund et al. 1996; Shattuck et al. 1997). Our current ventilator (Hedlund et al. 2000a,b) is shown schematically in Figure 3. The ventilator is controlled by a custom LabVIEW (National Instruments, Austin, Texas) application, operates in a Macintosh computer (Apple Computer, Cupertino, California), and uses a digital output board (National Instruments). This output then controls electropneumatic valves that pneumatically control a plastic breathing valve (Figure 4). The breathing valve attaches directly to the endotracheal tube of the animal to minimize ventilator dead space. This ventilator is an open system without any rebreathing of gases.

Because of the small size and internal volume of these breathing valves (Figure 4), the dead space of the valve is
Figure 6  (Right) Breathing valve (also in Figure 4) illustrates how the inspiration valve port (IN) opens to allow for inspiration, then closes momentarily; and how the expiration valve port (EX) opens for exhalation. The waveform plots on the left indicate the relation between the digital computer signal and valve ports that operate to control inspiratory, expiratory gas flow, and generation of imaging triggers. Reprinted with permission from Hedlund LW, Cofer GP, Owen SJ, Johnson GA. 2000a. MR-compatible ventilator for small animals: Computer-controlled ventilator for proton and noble gas imaging. Magn Res Imaging 18:753-759.

Figure 7  Macintosh display of our physiological monitor reveals the following: (a) electrocardiogram; (b) airway pressure waveform; (c) exhaled CO₂; (d) DC output pulse to trigger scanner; and cumulative records of (e) body temperature, (f) exhaled CO₂, and (g) heart rate. The ventilator control panel (h) is also evident. Ventilator and monitor applications can also run on PC computers. Reprinted with permission from Johnson GA, Turnbull DH, Fitzsimons EG, eds. 1999. In vivo microscopy: Technologies and applications. NIH Workshop for Small Animal Imaging, Gaithersburg, MD.
approximately 1.5 to 3% of the tidal volume, which is usually 3 to 6 cc for rats or guinea pigs. The length of hose needed to provide breathing gases from their sources is not critical in this design because the primary breathing control component is attached directly to the animal’s endotracheal tube. This feature is a potential advantage for any application where mechanical ventilation is needed but for which access to the animal is restricted by distance or space restrictions. This particular ventilator design also meets the much more stringent requirements for lung imaging with hyperpolarized gases, as discussed below.

Some of the breathing patterns that can be generated with this ventilator are shown in Figure 5. Each of these patterns has advantages for certain types of studies. The trigger generated from the ventilator computer (vertical hatched bar) is positioned at different points in the breathing cycle. Thus by using scan synchronous ventilation, imaging can be restricted to occur during end-expiration (1st and 2nd rows), when the lungs are at end-expiratory volume (functional residual capacity or FRC), or during long inspiratory gas flow (3rd line), during a short breath hold (4th line), or during longer breath holds equal to the duration of several breaths after a single full inspiration (last line). This last breath pattern of extended breath hold is particularly useful for MR spectroscopy experiments, when longer data collection times are needed (Möller et al. 2001).

These breathing patterns are computer generated by adjusting the timing of opening and closing of the inspiration and expiration valve ports on the breathing valve, as shown in Figure 6. In Figure 6 (right), the breathing valve and the operation of the individual gas ports can be seen—inspiration (IN) and expiration (EX). Also illustrated in Figure 6 (left) is how timing of opening and closing valve ports produces these breathing patterns. For example, for the normal pattern, the inspiration port opens for a preset duration, then closes shortly before the expiration valve port to allow exhalation. The various breathing patterns are created by changing the durations of inspiration and delays to the expiration port opening. The pair of valve ports for hyperpolarized (HP1) gas and air input are used for hyperpolarized gas imaging, as discussed below.

Physiological Monitoring for In Vivo Imaging

In vivo imaging studies with MR require anesthesia for which, to ensure safety and humane treatment, animals must be physiologically monitored and supported. In our laboratory, we maintain appropriate levels of anesthesia and support normal body temperatures with physiological monitoring. All of our basic procedures for imaging are noninvasive and thus allow us to perform survival studies. First, we anesthetize the animal with a very short-acting barbiturate, methohexital (45 mg/kg intraperitoneally), intubate perorally with a Quick Cath (Baxter, Deerfield, Illinois) cut to appropriate length, insert a rectal thermistor, and tape pediatric electrocardiogram electrodes to the paws. Next, anesthesia is maintained with isoflurane (1.5-3.5%) delivered with the ventilator. Electrocardiogram, airway pressure, exhaled CO2, body temperature, and heart rate are displayed on a computer monitor (Apple Computer, Cupertino, California) (Figure 7) using signal processors (Coulbourn Instruments, Lehigh Valley, Pennsylvania), an A/D computer board (National Instruments, Austin, Texas), and a LabVIEW (National Instruments) application. The monitor application also incorporates a feedback control loop for automatically maintaining body temperature (Qiu et al. 1997) by using warm air circulated through the bore of the imaging magnet. Also shown in Figure 7 is the front control panel for the ventilator, which operates in the same computer. It is essential to keep the small animal physiologically stable during the course of imaging if in vivo imaging is to be successful. During imaging sessions that may vary from a fraction of an hour to several hours, the physiological stability of the animal is maintained primarily in terms of anesthesia level, heart rate, and body temperature. The imaging examples described below utilize these physiological monitoring and control systems, as well as the ventilation methods previously described.
Examples of Scan Synchronous Ventilation for MR Imaging

One example of an MR image of an anesthetized, 300-g rat obtained by scan synchronous ventilation is shown in Figure 8. This image was acquired during end-expiration lung volume using cardiac gating and a very short echo delay time (345 μsec) projection sequence (Gewalt et al. 1993) that captures the very weak, short-lived signal from the lungs. The very rapid decay of the MR signal that occurs in the lungs, and not in other organs, is due to the lung’s high susceptibility resulting from the extensive network of gas-tissue interfaces. There is also a lack of signal from the airways, which is to be expected for structures devoid of protons. The lack of motion artifacts (ghosting) from the heart and lungs is due to the combination of projection imaging sequence, cardiac gating, and scan synchronous ventilation.

The importance of combined cardiac gating and scan synchronous ventilation is also seen in Figure 9, in which the heart of an anesthetized rat at six phases of the cardiac cycle is shown, based on use of a protocol similar to that used for Figure 8. The series of images were obtained at 20-msec intervals through the cardiac cycle starting from 1 msec after the QRS spike. In this example, the changes in left ventricular wall thickness and lumen diameter can be seen clearly, in addition to the alterations in diameters of various blood vessels (coronary, aorta, pulmonary) as they change over the course of the cardiac cycle.

An application of scan synchronous ventilation and cardiac gating in a model of lung disease is shown in Figure 10. Here we have used conventional spin echo imaging of four rats that were exposed to 85% oxygen at one atmosphere for 1 to 14 days. Within 1 day (A), there was little observable change from normal except increased peribronchial signal intensity; however, by days 4 (B) and 5 (C), there were significant pulmonary edema and pleural effusion that was completely reversed by day 7 (D). This example clearly

![Figure 9](image_url)
demonstrates how the sensitivity of MR imaging to protons of water can be used to advantage in this type of model of lung injury. In the next example (Figure 11), we instilled paraquat into the left lung of anesthetized rats and imaged them 1, 7, and 14 days later using a spin echo sequence with cardiac gating and scan synchronous ventilation. Paraquat or methyl viologen is known to produce an acute pulmonary edema and chronic fibrosis. Within 1 day (Figure 11A), there was evidence of edema by the presence of bright signal in the left lung, and within 7 days (B), partial reabsorption is revealed by reduction of the high signal area. Based on histological findings, fibrosis is present by 14 days. Edema was confirmed by wet/dry weight measurements. The unilateral injury also results in compensatory hyperexpansion of the contralateral normal right lung.

In all of the examples above (Figures 8-11), images were obtained using scan synchronous ventilation with the lung at end-expiratory volume. This is the lowest gas volume of the lung, which maximizes the proton signal and makes images maximally sensitive to detecting changes in water content of the lungs. However, in some instances, performing comparison imaging of the lung at two volumes, end-expiratory and end-inspiratory, can yield valuable pulmonary information, as described below, when performing hyperpolarized helium imaging of the lung.

**Hyperpolarized Gas Imaging of Pulmonary Airways**

A limitation of most conventional, noninvasive in vivo imaging methods for the lung is that they depend on either the

---

**Figure 10** Axial spin echo images from four anesthetized rats that had been exposed to 85% oxygen atmosphere for 1 (A), 4 (B), 5 (C), and 7 (D) days. The increased signal intensity in the lung parenchyma in B and C is evidence of pulmonary edema, and the homogeneous white band surrounding dorsal and lateral periphery of the lung is fluid of pleural effusion. Pixel size is 195 × 195 μm. This effusion is completely resorbed by 7 days (D). Reprinted with permission from Hedlund LW, Gewalt SL, Cofer GP, Johnson GA. 1996. MR microscopy of the lung. In: Cutillo A, ed. Application of Magnetic Resonance to the Study of the Lung: Armonk NY: Futura Press. p 401-415.
presence of tissue water or hydrogen (protons) or x-ray attenuating material. Most of the volume of the lungs, however, is composed of gases N₂, O₂, and CO₂ and water vapor, which provide poor substrates for imaging. Thus, most of the lung cannot be imaged directly by conventional methods, inasmuch as gas spaces of the lungs and extra pulmonary Airways are seen only as voids surrounded by the relatively low density of the lung parenchyma. However, MR imaging of the lung changed profoundly in 1994 when HP³He became available. When introduced into the lungs, this gas provides a rich MR signal source for directly imaging the lung’s gas spaces. A group of physicists at Princeton University, headed by William Happer, had previously developed a laser method for polarizing ³He and ¹²⁹Xe so that these gases could be used as signal sources for MR imaging (Albert et al. 1994; Happer et al. 1984; Johnson et al. 1998). Our laboratory was fortunate to work with this group, and together we produced the first live animal lung image with HP³He (Black et al. 1996). Hyperpolarized ³He has the advantage of being a MR signal source that is about 10 times greater than an equivalent number of protons typically involved in conventional MR imaging, and He is not readily absorbed by body tissues (Middleton et al. 1995). The gas is polarized by a laser process and not by a magnetic field. Thus, as soon as the HP³He polarization is used for imaging, the gas is no longer available as a signal source, unlike protons in conventional MR, which are depolarized by the magnet. An HP³He imaging session must use a fresh supply of polarized ³He, and imaging is therefore limited by the total amount of HP³He available. In our laboratory, this amount is usually about 1.5 l and requires about 10 hr to polarize to ∼30%. The total number of images that can be generated in an imaging session is dependent on the specific types of images, which can range from single slices to 3D data sets of the entire lung.

Given the limited supply of HP³He, it must be delivered very carefully to the animal. Several critical issues are involved in this process. Because the gas undergoes a natural depolarization in the reservoir before imaging use, it must be used within several hours. Contact with metals or oxygen hasten HP³He depolarization (Saam et al. 1995). We have incorporated features in the ventilator (Figure 3) to minimize unnecessary depolarization from oxygen and metals. For instance, HP³He is mixed with oxygen at the last possible moment at the start of inspiratory gas flow. In addition, the HP³He reservoir and tubing supplying gas to the breathing valve and breathing valve itself contain no metal parts (Hedlund et al. 2000a). These and many other issues of hyperpolarized gas imaging have been summarized recently (Kauczor 2000).

Examples of HP³He imaging are shown in Figure 12. On the left is a 5-mm-thick proton projection acquisition of the thorax of an anesthetized rat obtained during short breath holds at full inspiration. The lungs are present here as empty space devoid of signal. This example is the typical image of the chest showing the thoracic wall and vasculature. On the right is a 5-mm-thick slice from the same rat obtained with short breath holds at full inspiration of HP³He. Note the appearance of only the lungs and extrapulmonary Airways, that is, only the spaces occupied by the gas. The signal void areas around the bronchi and within the lung are spaces occupied by major and minor blood vessels, which are seen in the proton image (left). The lungs are seen without superimposed soft tissue structures or vasculature, in part because ³He is not absorbed into the tissue. We see
here a direct image of the intrathoracic gas spaces from the trachea to the very distal spaces consisting of terminal bronchioles and alveoli. Imaging, in this case, was synchronized by the ventilator computer to occur after the completion of a short inspiration of HP $^3$He during multiple short breath holds (Figure 6B). This example represents the most efficient way to use the very limited supply of HP $^3$He. It is possible to obtain registered images without moving the animal by using a dual frequency imaging coil-proton (83 MHz) and $^3$He (64 MHz).

With proper synchronization, it is possible to acquire images at different phases of the breathing cycle, as seen in Figure 13 (Chen et al. 1998). The vertical hatching in the small icons reveals when in the breath cycle the image was obtained. When image acquisition occurs during early inspiration, we are able to capture images of gas inflow before the lungs are completely filled with gas and, as a result, we are able to see the structure of the conductive airways more clearly. The exquisite detail of the airway tree are easily seen extending to approximately the 5th generation of branching. With the image at full inspiration (right), we can readily see the extent of complete filling of the lungs.

From early results with HP $^3$He, it was clear that $^3$He imaging of the lung could be a sensitive indicator of regional pulmonary ventilation. Application to the clinical realm warrants some caution because of the great flow rate and diffusion differences between helium and air; however, early results revealing ventilation defects in human disease are promising (de Lange et al. 1999; Kauczor et al. 1997). In a study with adult guinea pigs, we tested the sensitivity of HP $^3$He to detect intraluminal localized bronchial obstructions in the lung (Hedlund et al. 1997). Figure 14 reveals how we placed a catheter in the right or left mainstem bronchus for injection of a small amount of a fast-drying surgical cement to create a localized airway obstruction. On the right is an HP $^3$He gas image of the guinea pig airways obtained during a short period of early inspiration. Clearly shown in the right lung (image left) are the cranial, middle, caudal, and accessory lobe bronchi; and similarly in the left lung, we see the cranial, middle, and caudal lobe bronchi. Figure 15 comprises two examples of placement of the bronchial obstruction. In the first (top) example, images were obtained in early inspiration to reveal the fine detail of the bronchial tree, and the left caudal lobe bronchus was blocked, as can be seen from the image on the right compared with the preblockage image on the left. In the second (bottom) example, imaging was performed during several breath holds after full inspiration to detect any defects in filling of the lungs. The blockage was placed in the right caudal lobe bronchus. The postblockage image on the right reveals that gas flow to both the accessory and the right caudal lobes was obstructed.

It is clear that imaging the small animal lung with HP $^3$He is a very effective method for determining abnormalities of the gas flow and distribution in the lungs. We anticipate that this method will also be valuable in studies relating to airway constriction and dilatation in such models as asthma.

In another example of a model of lung disease, we used HP $^3$He to examine an elastase model of emphysema in the rat (Chen et al. 2000). Elastase instilled into the trachea...
Figure 13  Hyperpolarized $^3$He images of an anesthetized guinea pig revealing airways during early inspiration (left) and full extent of gas filling (right). Reprinted with permission from Chen XJ, Chawla MS, Hedlund LW, Möller HE, MacFall JR, Johnson GA. 1998. MR microscopy of lung airways with hyperpolarized $^3$He. Magn Res Med 39:P79-84.

Figure 14  (Left) Method of inserting a catheter through the side of the endotracheal tube and then into the right or left mainstem bronchus to the level of caudal lobe. (Right) Airway structure from an anesthetized guinea pig.
results in the breakdown of the elastic components in the tissue adjacent to the airspaces and an overall enlargement of alveolar volume. Our study was based on the phenomenon that changes in the diffusion of $^3$He can be used to detect changes in the microstructure of the lungs. By using diffusion-sensitive MR imaging, it is possible to measure the apparent diffusion coefficient (ADC) of $^3$He. The term apparent is used because the mobility of the gas is really a function of the microstructure that restricts the true diffusion of the gas. This measurement can be used in turn to determine the volume available for $^3$He diffusion. For instance, ADC of $^3$He in the trachea, where its diffusion is

---

**Figure 15** (Top) Hyperpolarized $^3$He images obtained during early inspiration, and (bottom) during multiple short breath holds after full inspiration. In each example, the left image reveals the lungs before bronchial obstruction and the right, after obstruction is placed. In the top pair, the result of placing an obstruction in the left caudal lobe is evident. In the bottom pair, the effect of blockage in the right caudal lobe bronchus is shown. In this case, gas flow to both the right caudal and accessory lobes is blocked. Reprinted with permission from Hedlund LW, Chen XJ, Chawla MS, Cofer GP, Cates G, Happer W, Wheeler CT, Johnson GA. 1997. Pulmonary airway obstruction in an animal model: MRI detection using hyperpolarized $^3$He. ISMRM 5th Scientific Meeting, p. 183.
relatively unrestricted, is 2.4 cm²/sec; whereas in the gas exchange region of the normal lung in the alveolar spaces where ³He diffusion is severely restricted, the ADC is 0.16 cm²/sec (Chen et al. 1999b). Based on these measurements, we reasoned that microstructural changes in the alveolar spaces of the lung due to an emphysema-like condition would be reflected in a change in ³He ADC. We expected that as these spaces were enlarged by treatment with elastase to mimic panacinar emphysema, the ³He ADC would be increased. To test this hypothesis, we treated rats with elastase to reduce the elastic components of the lungs and then imaged the animals 4 wk later. We compared the ADC of ³He in the lungs of normal and elastase treated animals at end-expiratory volume and during a breath hold after full inspiration. The normal lung exhibited a significant reduction in ADC from full-inspiratory to end-expiratory volume, as would be expected for a normal lung. In other words, the apparent diffusion of ³He at full inspiration, when alveoli are fully expanded, is much greater than the ADC at end-expiratory volume, when alveoli are at their smallest volume. However, in the elastase-treated animals that suffered a loss in elastic properties, there was little difference between the full-inspiration and end-expiration ADC, indicating that the alveoli at end-expiratory volume remained nearly fully expanded because of the damage to the elastic components of the lungs. Conventional histological examination of the lung in each case confirmed the extent of damage in the treated lungs and the normal condition of the control animals. In this case we used HP ³He as a tool for quantitatively assessing changes in the lung by measuring ADC and these changes were detected independent of visually detectable changes in the lung images. It would not have been possible to perform these studies without the use of HP ³He and the ability to control image data acquisition at two different lung volumes.

Using computer-controlled ventilation (Hedlund et al. 2000b), we are able to control the delivery of HP ³He to the animal carefully and to synchronize image data capture at any phase of the breathing cycle. In Figure 16 are shown HP ³He images from an anesthetized guinea pig obtained by capturing imaging data in a series of 100-msec intervals starting at the beginning of inspiration (Viallon et al. 1999). MR imaging utilized a special radial acquisition in a CINE mode, which collects image data dynamically and allows observation of the inflow of HP ³He into the lungs. Figure 16a reveals the summation of the images over the entire 800-msec acquisition. In the eight images that follow and represent 100-msec intervals from the beginning of inspiration, it is possible to visualize the HP ³He at the beginning of inspiration (b), as it moves from the extrapulmonary airways in to the individual lobar bronchi (c), and finally to the most distal gas exchange regions (d-g) at the end of inspiration. The distribution of gas during the short breath hold can be seen in 16h, and the beginning of exhalation can be seen in 16i. Similarly, it is possible to observe inspiratory gas flow in the axial view in 50-msec intervals of early inspiration (Figure 17). With this kind of imaging and gas delivery control, it will be possible to perform dynamic pulmonary function analysis in the small animal, measuring regional gas flow velocity and volume and evaluating how these may be changed by drug treatments in models of lung
disease. Such regionally specific information is not currently available with conventional pulmonary function tests that provide only global assessments.

The limits of spatial resolution for proton and HP $^3$He imaging are not clearly known; however, by using both together, we may have sufficient spatial and contrast resolution to image the smallest structural units of the lung—alveoli or a small collection of them. Early thoughts on the limits of spatial resolution of HP $^3$He imaging suggest that the gas images would have poor resolution because of the high diffusion rate of this $^3$He in free space. However, as previously documented (Chen et al. 1999a), the apparent diffusion coefficient of $^3$He in the structurally restricted spaces of the lung is much lower than in unrestricted spaces. This knowledge has led to a reassessment of the lower limits of resolution with $^3$He and, as can be seen in Figure 18, exceptional spatial detail in the anesthetized rat lung using $^3$He. Registered proton images from this same study can be seen in Figure 19. We believe these images are the highest resolution lung images with HP $^3$He yet obtained (Johnson et al. 2001): voxel size is $117 \times 117 \times 468 \mu m$. These images clearly reveal helium within alveoli, that is, at the very edge of the lungs. However, at this time, we cannot claim to have resolved the individual alveolus, which is on the order of $100 \mu m$ in diameter in the rat. The ability to see registered HP $^3$He and proton images at these high resolutions will no doubt be useful in future work in critically examining the morphological changes occurring in vivo in small animal models of pulmonary disease.

Conclusions

In this brief review we have confirmed that it is possible, with a combination of techniques, to perform high-quality in vivo MR imaging of the small animal lung, both in normal and disease models. The problem of lung motion can be

Figure 18 Six selected coronal slices from a three-dimensional data set from an anesthetized rat lung using HP $^3$He. The most intense signal structures here are conductive airways. Areas devoid of signal within the lung are spaces occupied by blood vessels. Acquisition time for this set was approximately 20 min. Proton images at the same coronal levels are shown in Figure 19. Reprinted with permission from Johnson GA, Cofer GP, Hedlund LW, Maronpot RR, Suddarth SA. 2001. Registered $^1$H and $^3$He magnetic resonance microscopy images of the lung. Magn Res Med 45:365-370.
resolved by synchronizing image acquisition to the breathing cycle. Furthermore, the effect of low tissue density of the lung can be minimized by imaging during the time of the highest lung density, at end-expiratory volume. The structural characteristic of the tissue-gas interfaces of lung resulting in MR high susceptibility and weakening the already poor proton signal can be resolved by using a projection sequence with a very short echo time. Finally, the gas spaces of the lung can now be imaged directly using hyperpolarized helium in the breathing mixture. Thus, with the proper tools, the small animal lung can be studied dynamically in vivo in survival studies. This capability creates many possibilities for basic studies of the lung and the development of better methods for small animal pulmonary research.

**Acknowledgments**

We thank the many researchers who have contributed to the work described here (see cited references) and our funding agencies (National Institutes of Health, National Center for Research Resources [P4105959], and National Heart, Lung, and Blood Institute). We also convey special thanks for many years of help from Ted Wheeler for animal support and Elaine Fitzsimons for manuscript preparation.

**References**


